An Attempt to Hybridize *Drosophila* Species Using Pole Cell Transplantation

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ABSTRACT

We have made hybrid embryos in *Drosophila* by pole cell transplants, by transfering pole cells from two species, *D. rajasekari* and *D. eugracilis*, into sterile *D. melanogaster* hosts. These females were then mated to *melanogaster* males and the older these females were, the further their hybrid offspring developed. In the case of the *rajasekari/melanogaster* hybrids, the embryos form cuticle but had defective heads, while the *eugracilis/melanogaster* hatched as larvae that grew but did not moult to the second instar. Hybrid pole cells could be transferred to *melanogaster* hosts but they failed to make eggs.

AS species evolve, they change in pattern, shape and proportion (*e.g.*, VAL 1977). Any understanding of the genetic basis of these changes should also illuminate how pattern and form are determined in any one species.

Our plan was to take species of *Drosophila* that differ significantly in form and bring large pieces of their genome for analysis into that of *melanogaster* by making hybrids. We hoped to overcome the reluctance of different species to mate (BOCK 1984) by transplanting the germ cells between species so that *melanogaster* females would be the unwitting carriers of alien germ cells (SANTAMARIA 1977). We did not expect the F_1 hybrids to develop far, but thought they might well produce viable germ cells that could then be transplanted into surrogate *melanogaster* hosts again and thereby backcrossed to *melanogaster*, diluting the alien genome further. This process could, we hoped, be repeated until we achieved fertile hybrids consisting mainly of *melanogaster* but with pieces of the genome of the alien species. Some of these pieces might produce changes in the pattern of *melanogaster*, that could then be analyzed by standard genetic and molecular methods. The plan is a lofty one and, like most such plans, failed to achieve its objective. However, we have made hybrids of two species of *Drosophila* (*D. eugracilis* and *D. rajasekari*) with *melanogaster* and there may be some lessons of use to others.

METHODS

We use standard methods of pole cell transplantation (VAN DEUSEN 1976) except that we prefer to dry the host eggs a little less, withdraw and discard some cytoplasm, before transplanting the pole cells. This technique improves survival and reduces ejection of the pole cells which can often occur in the hour following the transplantation. Pole cells from one donor were split between two or three hosts. The host eggs arose from a cross between *D. melanogaster white*$^+$ males carrying the dominant female sterile mutation *ovo*^{D1} (BUSSON *et al.* 1983) and *white* females. Females carrying *ovo*^{D1} lay no eggs but make excellent hosts for implanted pole cells. (In *ovo*^{D1}*/white* females a mitotic recombination in the germ line can eliminate the *ovo*^{D1} mutation, but, such recombinations are rare in the absence of X-rays (BUSSON *et al.* 1983); one female from about 200 surviving the transplantations laid a few fertile eggs, which progeny tests established were derived from such a mitotic recombination). The surviving host females were crossed to *In(1)AB melanogaster* males. *In(1)AB* rescues *simulans/melanogaster* hybrid males (HUTTER, ROOTE and ASHBURNER 1990) and we thought its presence might help.

The strategy was to generate enough egg laying females of the first or T1 generation (see Figure 1) so that their hybrid embryos could be used as donors to transfer pole cells to surrogate mothers of the next generation. Since donor eggs have to be of a defined age and since each female, at best, lays only a few eggs an hour, at least 10 fertile females are necessary to make the experiment practicable. Since donor eggs must be female it is not possible to achieve more than 50% fertility in the surviving females. In practice we usually achieve about half that proportion (*e.g.*, LAWRENCE, JOHNSTON and STRUHL 1983).

The T1 generation males, which probably would have contained sperm derived from the alien species, were crossed *en masse* to *white D. melanogaster* females and the progeny optimistically screened for red eyed hybrids. None were found.

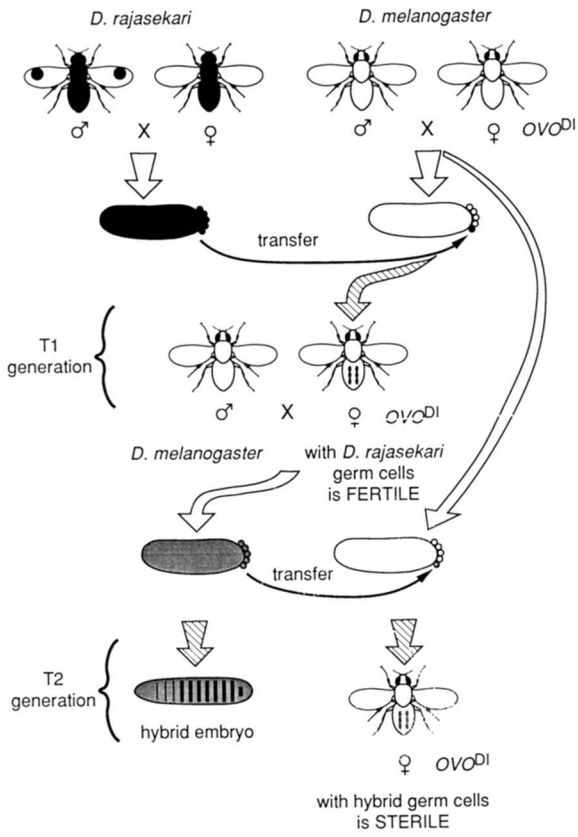

FIGURE 1.—A summary of the experiments.

RESULTS

We chose two species that were markedly different in form and pattern from *melanogaster*, but were reasonably closely related to it. Both species are members of the *melanogaster* species group: *D. eugracilis* is in a separate subgroup of its own and *D. rajasekari* belongs to the *suzuki* subgroup (BOCK and WHEELER 1972). The conspicuous black spot on the wing of male *rajasekari* makes it particularly appealing, but both species display many differences in pattern, especially in their sex comb teeth and in their pigmentation (REDDY and KRISHNAMURTHY 1968; BOCK and WHEELER 1972).

***D. rajasekari*:** The entire experiment was taken through two cycles. In the first cycle 40 T1 females were produced, of which 10 were fertile on being crossed to *melanogaster* males. This result shows that the germline of *rajasekari* is able to develop well in the soma of *melanogaster* and make eggs that can be fertilized by *melanogaster* sperm. These hybrid zygotes developed very poorly but as the T1 females aged, they developed further and further. In the first week the embryos failed to form blastoderms and the polar buds made spheres of cytoplasm that lacked nuclei. In embryos from older females, blastoderms were formed and some even formed cuticles. The cuticles had poorly developed terminal structures, especially at the anterior end, but the main trunk shows a well formed pattern of segmentation. Of thousands of eggs laid over the lifetime of the T1 females (about 1 month) none hatched. Embryos laid by the older females developed more complete pole cells, these were small cells and contained small nuclei. The second generation transplantations produced 45 T2 females, none of which laid any eggs.

In the second attempt we produced about 25 fertile T1 females and made a stalwart effort to transplant the pole cells from the progeny of these. Again, the older females produced better embryos with less abnormal pole cells. From these transfers we obtained 90 T2 females which were crossed to *melanogaster* males; in all they laid only 5 eggs, none of which developed. All the 35 females still alive after a month were dissected and all but one had no ovaries. One had bilateral ovaries with some developing eggs and we presume was responsible for the 5 eggs laid. It seems possible, but not certain, that these inanimate eggs came from the hybrid germ cells.

***D. eugracilis*:** From the transfers we obtained many fertile T1 females, showing that *eugracilis* germ cells are able to grow and develop in the *melanogaster* hosts. Moreover, these females, when mated to *melanogaster* males, laid eggs that developed well and many hatched to give active larvae (of one sample 54/171 hatched). Some few of these survived to grow considerably and some were still alive as long as 10 days after hatching–but none we examined had moulted to the second instar, and of many thousands of hybrid eggs, not one pupated. The cuticle pattern of these larvae was well developed and showed characters inherited from both parents (Figure 2).

As with the *rajasekari* case, the young females laid hybrid eggs that developed poorly but, as they aged, their progeny developed more and more normally. Initially the pole cells looked small and depauperate, but later embryos carried full-size typical pole cells with nuclei. These were transferred in a series of experiments and more than 100 T2 females obtained. These were kept and between them they managed to lay one egg, which failed to develop at all. Later, as the females began to die of old age, 35 were dissected and one unilateral ovary was found with a few eggs in it.

DISCUSSION

There are two main points of interest in these results. First, the germ line and soma of the two pairs of *Drosophila* species are able to collaborate and make viable hybrid zygotes. Even though they do not develop beyond the first stage larvae, they differentiate well. This is typical of hybrids made between species in different subgroups (KAMBYSELLIS 1968). Second, the hybrid germ cells are incompetent. If they divide at all, and this must be considered questionable, the

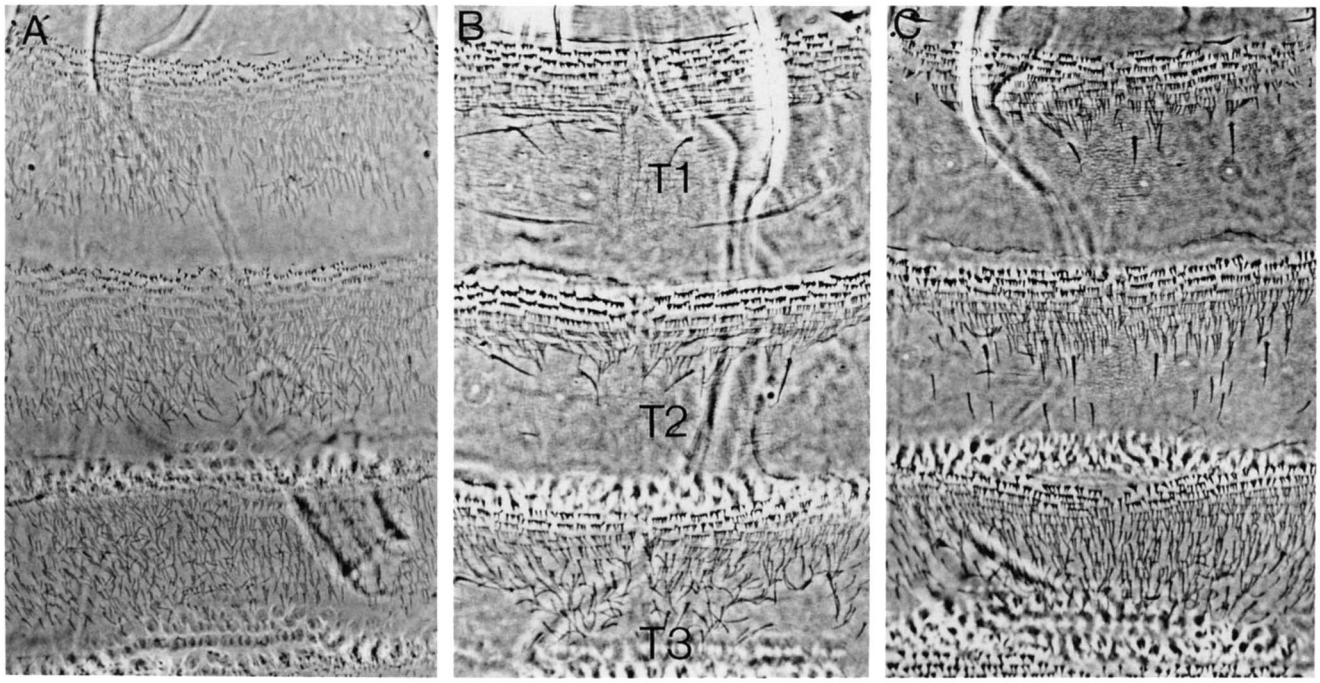

FIGURE 2.—Dorsal cuticle of the thorax of first instar larvae, phase contrast. (A) *D. melanogaster*, (B) *D. eugracilis* and (C) *melanogaster/eugracilis* hybrid, which is similar to *eugracilis* but with some additional denticles. T1–T3 are the three thoracic segments.

eggs they rarely produce are inviable. We consider these points in turn.

Soma and germ line: There is much molecular conversation between the developing oocyte and the surrounding follicle, particularly in defining the terminal regions and in positioning the dorsoventral axis of the embryo (reviewed in ST. JOHNSTON and NÜSSLEIN-VOLHARD 1992; LAWRENCE 1992). It is clear from our results that these interactions are effective when the conversation is between *melanogaster* soma and *eugracilis* germ cells–because the hybrid larvae appear to be perfectly formed–even though *D. eugracilis* and *melanogaster* have been evolving separately for tens of millions years. However, the rare hybrid larvae coming from crossing *rajasekari* and *melanogaster* have normal dorsoventral patterning but defective heads and tails, which suggests some problems in the terminal system–where it is known that a signal is passed from the oocyte to the follicle and, eventually, back again.

In both experiments, where the soma and the germ line are from different species, the development of the hybrid zygotes is dependent on the mother's age. The older females lay fertilized eggs that look more normal and develop further. Similarly, in hybrid dysgenic crosses with the *IR* system, the hatching percentage from dysgenic mothers improves from 0 to 80% as the mothers age (PICARD *et al.* 1977). It may be that the result of an interactive event between the cells of the two species is gradually "diluted" as the germ line stem cells divide. In P-M dysgenesis the germ cells begin to degenerate early in development–as they migrate toward the incipient gonad (NIKI and CHIGUSA 1986).

Viability of hybrid germ cells: SANTAMARIA (1977) investigated the cause of sterility in *melanogaster/simulans* and *melanogaster/mauritiana* hybrids by transplanting pole cells. He showed that the hybrid somas were able to support the growth of *melanogaster* germ cells, suggesting that the sterility is entirely due to incompatibility within the hybrid germ cells themselves. There is other evidence that hybrid germ cells are less capable of growth and development than hybrid somatic cells (SANCHEZ and SCHMID 1984). Here, we provide more: In neither of the two hybrids can the germ cells divide and develop even though in both cases the somatic cells can construct a well differentiated larva. In the *melanogaster/eugracilis* hybrids the somatic cells divide and differentiate, giving perfectly ornamented larvae that are able to move, feed and grow.

We can only speculate about the reasons for this. One study (ORR 1992) of the fourth chromosome of *simulans* that confers hybrid male sterility has shown that only a very small region, probably a single gene, is responsible. Likewise, the effects of single mutations on chromosome *1* in *melanogaster* (HUTTER, ROOTE and ASHBURNER 1990) suggests that hybrid viability may be due to a small number of genes of large effect.

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